24

Plasmodium fragile Dissanaike, Nelson, and Garnham, 1965

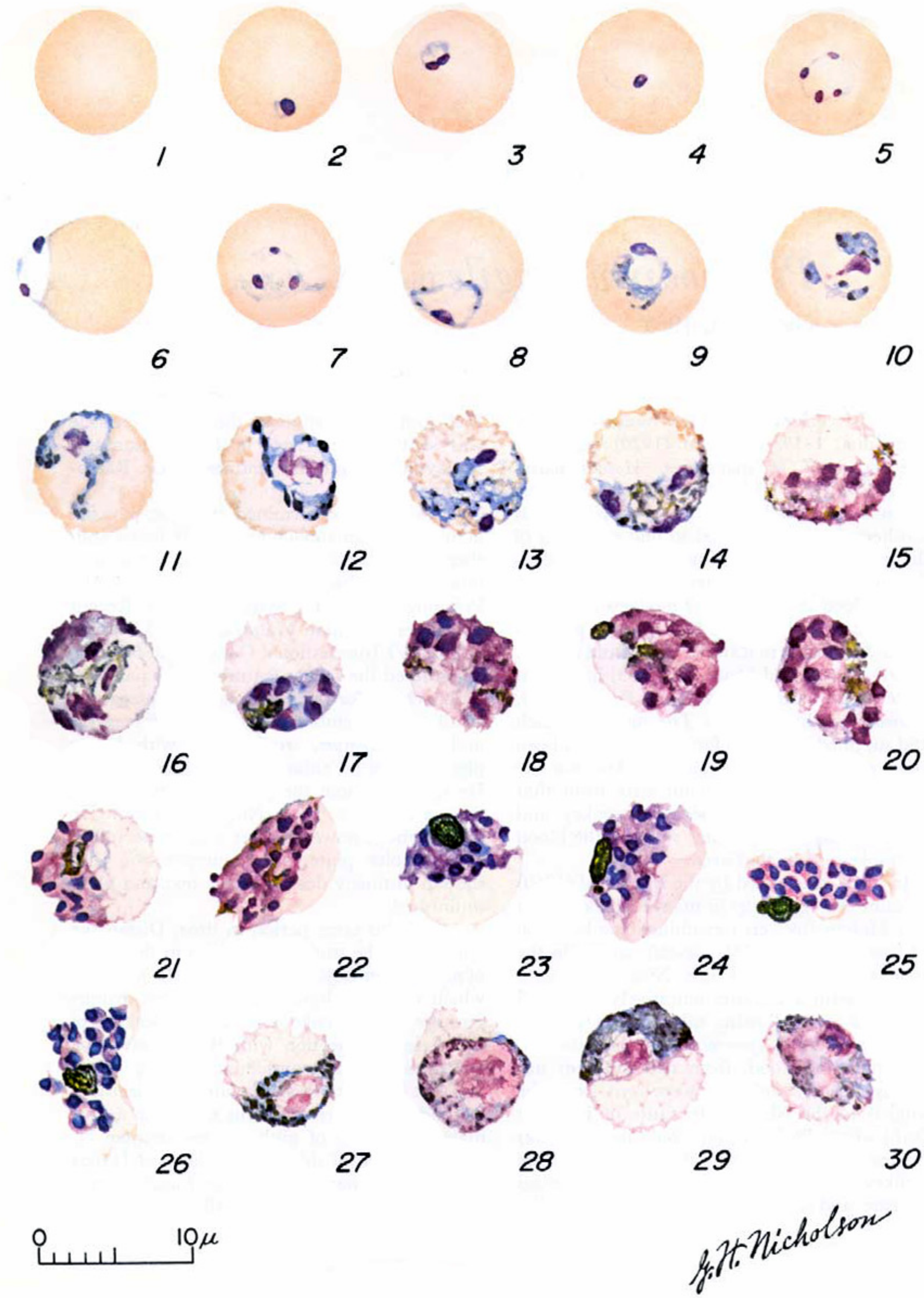
DURING the months of May and June, 1919, Donovan (1920) examined the blood of 76 macaques, *Macaca sinicus* (= *radiata)* and 10 langurs *(Presbytis priamus)* taken in the valleys of the Nilgiri hills in southern India, but failed to find examples of the genus *Plasmodium.* However, in an addendum to the paper, he mentioned that a slide of the blood of a *M. sinicus* monkey had been sent to him and on it he had found a plasmodium. According to Ramakrishnan and Mohan (1961), Sinton and Mulligan (1933) had given a tentative identification of *P. inui* var. *cynomolgi* to this parasite. The monkey which had supplied the blood for the smear had been taken at an altitude of 4,000 feet. This was the first instance of malaria not only from that area but also from that species of monkey, and Donovan suggested further study of the blood of monkeys from that area.

In 1960, stimulated by the Eyles *et al* (1960) account of *P. cynomolgi* in man, Ramakrishnan and Mohan (loc. cit.) examined the blood of 13 brown monkeys *(M. radiata)* caught in the area of Kallar, Nilgiri hills. Nine were found infected with a parasite tentatively identified as *P. inui* and, following splenectomy, a parasite resembling *P. cynomolgi* appeared also.

Samples of blood, from two different naturally infected animals, were sent from the Nilgiris to the Malaria Institute of India at Delhi where Prakash and Chakrabarti (1962) studied infections in a total of 241 *M. mulatta* monkeys; 124 were infected with the cynomolgi parasite and 117 with the inui-like parasite. Following these studies, the latter parasite was sent to Dr. Eyles in Kuala Lumpur, Malaysia, through the kindness of Dr. Ramakrishnan.

Eyles (1963) determined that the parasite actually had an asexual cycle of 48 hours and, therefore, a tertian rather than quartan periodicity; and, that it was a new species. At the Washington, D. C. Symposium on Recent Advances in Simian Malaria, in 1963, as part of the XVI International Congress of Zoology, he outlined the salient features of the parasite: its penchant for deep circulation schizogony, small rings resembling *P. coatneyi, P. knowlesi,* and *P. falciparum,* trophozoites with heavy pigment and no enlargement of the host cell. He did not name the parasite, preferring to discuss it as the "New Nilgiri Parasite." He did mention, however, that a full description with a color plate was in preparation. His sudden untimely death left the text and plate unfinished.

About this same period in time, Dissanaike had begun the study of parasites in the blood of monkeys in Ceylon, and among the malarias which came to light was the "New Nilgiri Parasite" (Dissanaike *et al,* 1965). Because of Dr. Eyles' connection with the parasite, the Dissanaike group considered naming it for him but by the time they made their decision, we had already reserved his name for a new malaria parasite of gibbons (see chapter 7). When advised of this, Dissanaike *et al* (1965) proposed the name *Plasmodium fragile* to emphasize its effect on the host cell.



PLASMODIUM FRAGILE

Cycle in the Blood
PLATE XLVIII

The youngest forms are delicate rings which may occur as a multiple infection in the host red cell. The older rings may display an accessory chromatin dot (Fig. 3) or the chromatin bodies may number up to 3 or 4 (Figs. 7, 5). As the parasite grows, it develops a prominent vacuole, becomes amoeboid, and exhibits numerous large pigment granules which are scattered throughout the cytoplasm. The pigment is black with a prominent yellow sheen (Figs. 11, 12) and occurs as irregular blocks or heavy spherical bodies in marked contrast to the rice grain-like pigment in *P. coatneyi.* Just preceding schizogony, the parasite occupies a large portion of the host cell which may show some alteration marked by pallor and distortion of the periphery but without enlargement.

The schizonts ordinarily do not fill the host erythrocyte and generally lie to one side of it (Figs. 14-17). The merozoites fragment from the main mass and, in the mature schizont, may number up to 18 or 19 with an average number of about 16. Toward the end of schizogony, there is marked host cell distortion. The pigment accumulates in a bulky mass but retains its black yellowish sheen (Figs. 24-26).

Gametocytes are generally abundant in developed infections. The macrogametocyte is generally oval to spherical with a prominent eccentrically located red nucleus. The pigment appears shattered or disintegrating (Figs. 29-30). The tendency toward oval gametocytes is an intriguing feature in the light of its close relationship to *P. coatneyi* and therefore to *P. falciparum.* The microgametocytes may assume irregular shapes but always display a prominent, usually diffuse, red staining nucleus with a prominent 'karyosome' area. The pigment is fragmented and dispersed in the cytoplasm (Fig. 28).

The parasite has an asexual cycle of 48 hours.

Sporogonic Cycle
PLATE XLIX

Eyles (loc. cit.) got oocyst development of the Nilgiri parasite in *Anopheles maculatus* on one occasion. Mosquitoes were fed on an intact *M. mulatta* monkey infected by the inoculation of parasitized blood. On the 10th day of patent parasitemia, when the parasite count was 17 per 100 red blood cells, a lot of *A. maculatus* mosquitoes was allowed to feed. Each of 16 mosquitoes dissected had oocysts (6.5 per gut) but three salivary glands examined 13.5 days after feeding failed to demonstrate sporozoites. Dissanaike *et al* (1965) tried to infect *A. maculipennis atroparvus* and *A. aztecus* with *P. fragile,* incubating the first group of mosquitoes at 27° C and the second group at the same temperature for 24 hours and then at 20° C. No development took place in either situation.

In our hands, *P. fragile* has been a difficult parasite with regard to mosquito infection. We eventually found that infections could be obtained if *M. mulatta* monkeys with chronic infections were splenectomized and mosquitoes then allowed to feed between the 2nd and 10th day after splenectomy. Moreover, infection of mosquitoes generally occurred on the days of high parasitemia when the predominant parasites in the peripheral blood were small rings.

The sporogonic cycle has been studied in two species of mosquitoes, *Anopheles freeborni* and *A. b. balabacensis.* Observations began 5 days after feeding and continued through day 17. Extrinsic incubation took place at a temperature of 25° C.

PLATE XLVIII.—*Plasmodium fragile*.

Fig. 1. Normal red cell.
Figs. 2-7. Young trophozoites.
Figs. 8-10. Growing trophozoites.
Figs. 11-12. Mature trophozoites.
Figs. 13-22. Developing schizonts.
Figs. 23-26. Nearly mature and mature schizonts.
Figs. 27-28. Immature and mature microgametocytes.
Figs. 29-30. Mature macrogametocytes.

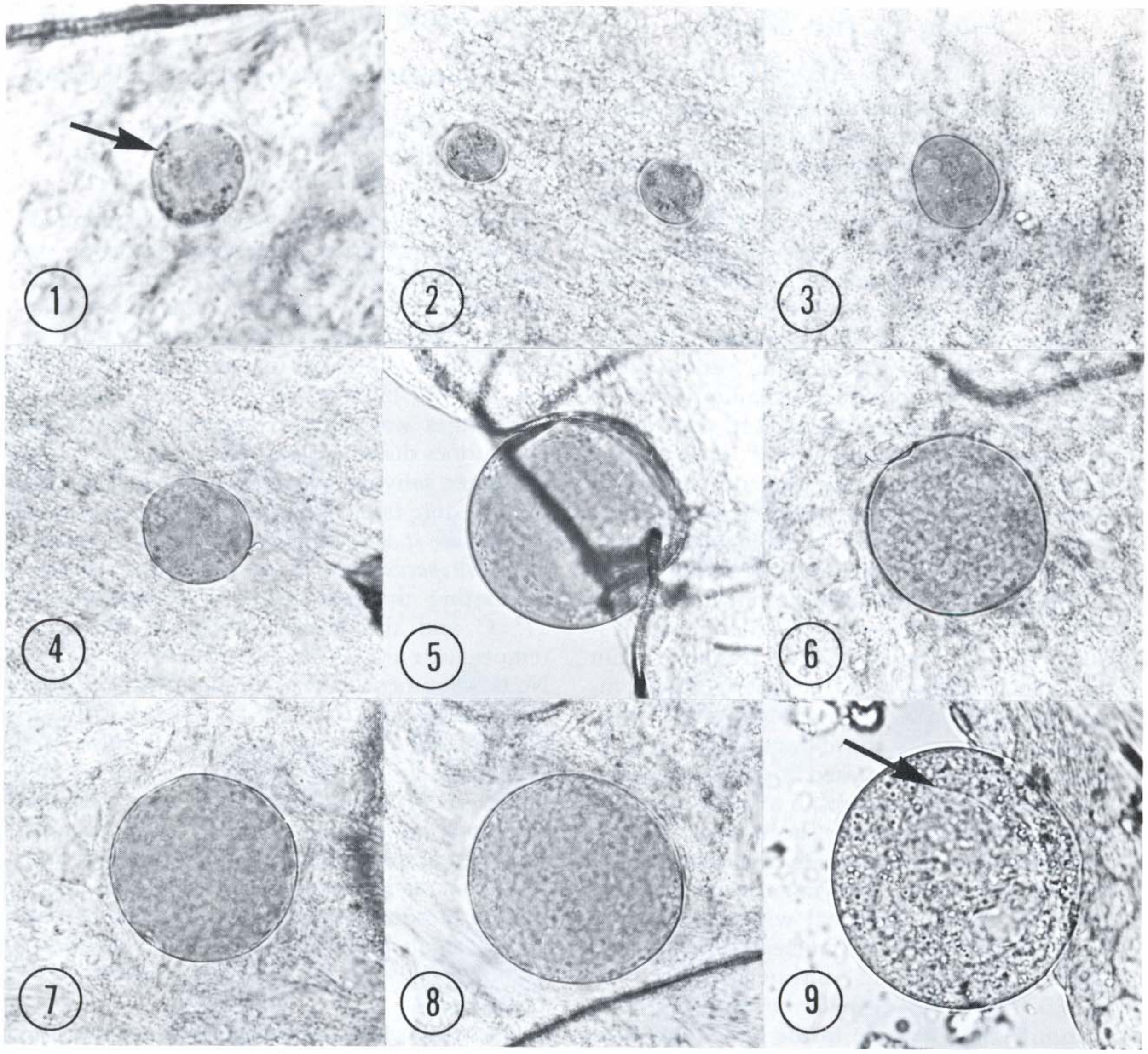


PLATE XLIX.—Developing oocysts of *Plasmodium fragile* in *Anopheles freeborni* mosquitoes. X 580 (Except Figure 1.).
Fig. 1. 6-day oocyst showing peripheral arrangement of pigment. X 1300.
Fig. 2. 8-day oocysts.
Fig. 3. 9-day oocyst.
Fig. 4. 10-day oocyst.
Fig. 5. 11-day oocyst.
Fig. 6. 12-day oocyst.
Fig. 7. 13-day oocyst.
Fig. 8. 13-day oocyst.
Fig. 9. 14-day oocyst showing early differentiation.

The results of the oocyst measurements are presented in Table 38. In *A. freeborni,* at day 6, the mean oocyst diameter was 10.8 μ with a range of 9 to 12 μ. The oocysts continued to grow so that on day 16, they had an average diameter of 58.7 μ with a range of 35 to 77 μ. Sporozoites were first seen in the salivary glands on day 16.

Measurement of the oocysts in *A. b. balabacensis* at day 5 gave a mean diameter of 9.8 μ with a range of 8 to 12 μ. The oocysts in this mosquito were quite similar to those seen in

A. freeborni except that on day 17, oocysts having diameters of up to 100 µ were seen. Sporozoites were present in the salivary glands on day 16.

A comparison of the growth rate of *P. fragile* and *P. cynomolgi* in *A. freeborni* mosquitoes (Fig. 67), indicates that this parasite takes approximately 16 days to complete its

TABLE 38.—Oocyst diameters *of Plasmodium fragile* in *Anopheles freeborni* and in *A. b. balabacensis.*

Days after Infection	*A. freeborni*			*A. b. balabacensis*		
	No.	Range*	Mean	No.	Range	Mean
5				17	8-12	9.8
6	15	9-12	10.8			
7	48	8-18	13.1	25	12-19	15.9
8	39	12-27	15.8	64	9-21	16.2
9	27	14-35	23.8	100	11-52	22.3
10	63	13-37	23.0	8	20-37	28.5
11	41	18-53	32.9	42	18-46	29.9
12	43	21-52	37.5			
13	31	25-55	41.9			
14	25	39-73	55.6†	2	50-64	57.0
15	14	41-77	61.5†			
16	24	35-77	58.7†**			**
17				39	30-100	60.4†**
Totals	372	8-77		297	8-100	

* Measurements expressed in microns; incubation temperature was at 25° C
† Oocyst differentiation.
** Sporozoites present in the salivary glands.

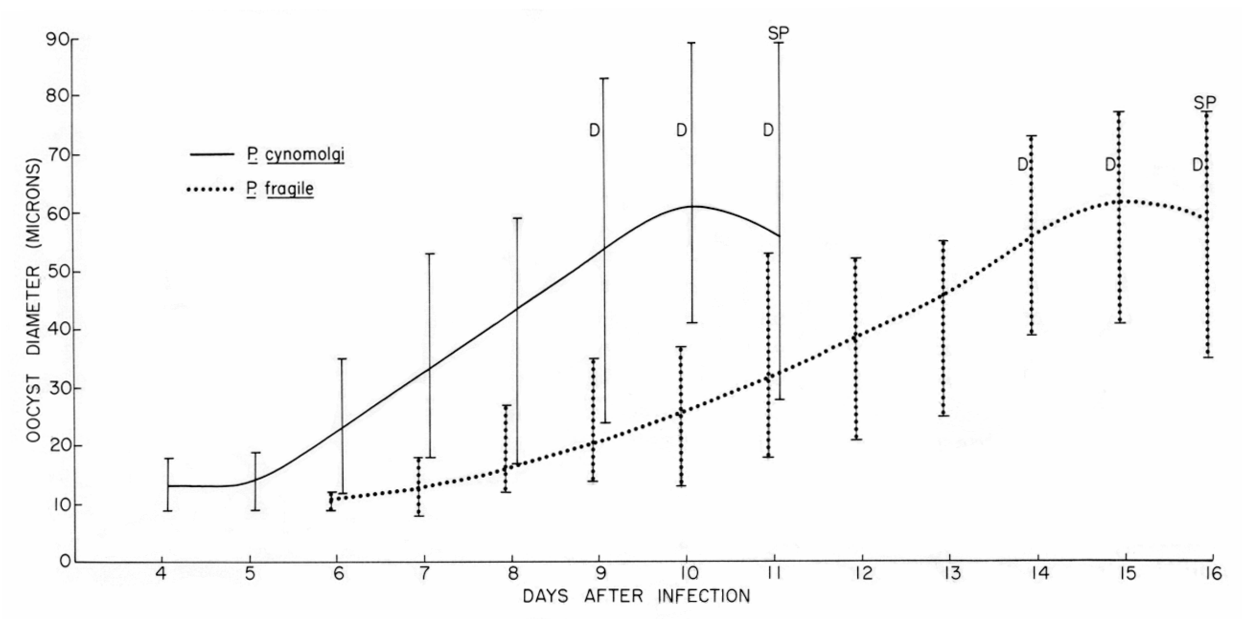


FIGURE 67.—Mean oocyst growth curves and ranges in oocyst diameters of *Plasmodium fragile* and *P. cynomolgi* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

development versus 11 days for *P. cynomolgi. Plasmodium fragile* is probably the slowest growing primate malaria with tertian periodicity.

The sporozoites in *A. b. balabacensus* were infective as shown by their ability to initiate infection in three intact *M. mulatta* monkeys. The prepatent period was 17 days in each animal.

Cycle in the Tissue

There are no data on the exoerythrocytic cycle of this parasite.

Course of Infection

Three different groups of investigators had studied this parasite in different hosts before it came into our hands through the kindness of Prof. P. C. C. Garnham. None had actually described the course of the infection either in the normal host or in the rhesus monkey which supports its growth well.

We were able to follow the course of the infection of 18 *M. mulatta* and 2 *M. fascicularis* monkeys (Fig. 68). Fifteen rhesus monkeys received their infection via parasitized blood and three via the inoculation of sporozoites. Following the passage of parasitized blood, the parasitemia rose to a level of approximately 240,000 per mm^3 by day 10 and declined slowly to a low of approximately 1,000 by day 40 and thereafter exhibited a secondary rise in parasitemia. Of the 15 animals, 5 died 8 to 12 days after inoculation (mean 10.5 days). In addition, one of three animals inoculated with sporozoites did not survive the infection (Fig. 69) giving a mortality rate of 33.3 percent. The two *M. fascicularis* monkeys had lower though persistent parasitemias during a 60-day period of observation. The pattern of deep circulation schizogony, marked by alternate days of high and low parasitemia, is illustrated in Fig. 69.

Host Specificity

The normal hosts of *Plasmodium fragile* are *Macaca radiata* (Ramakrishnan and Mohan, 1961) and *M. sinica* (Dissanaike *et al,* 1965). It has been shown experimentally to be infective to *M. mulatta* and *M. fascicularis.* Dissanaike, *et al* (1965) inoculated three men intramuscularly with blood containing *P. fragile*--no infection resulted during an observation period of one month. We have made two attempts to transmit the infection to man by the bites of *A. b. balabacensis* mosquitoes but no infection

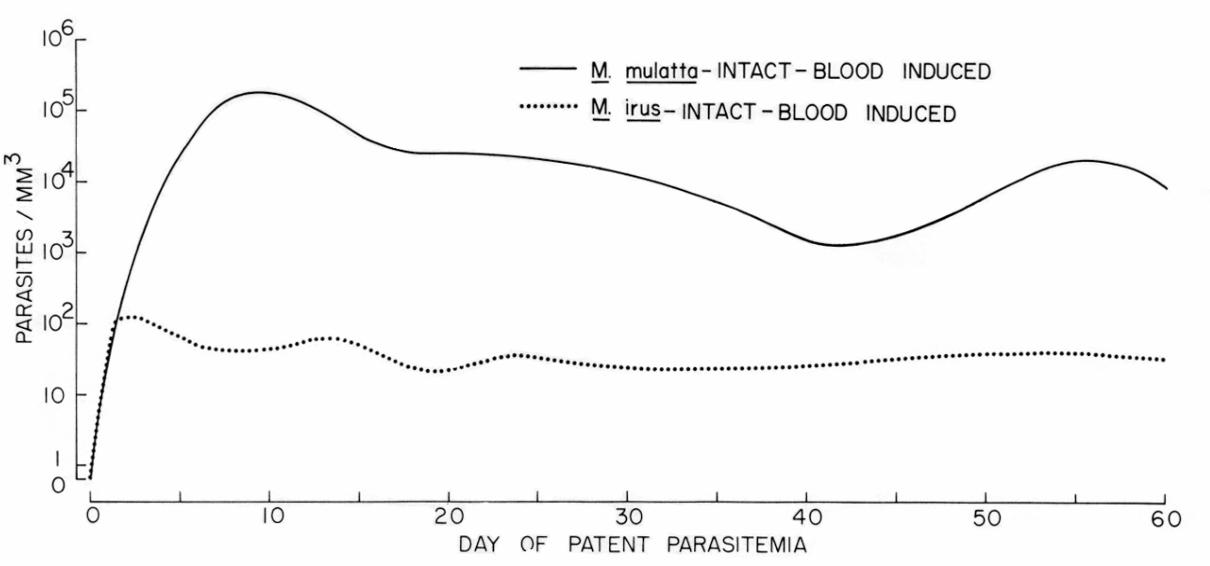


FIGURE 68.—Median parasitemia curves of *Plasmodium fragile* in 15 *Macaca mulatta* and 2 *M. irus* (= *fascicularis*) monkeys infected by the inoculation of parasitized blood.

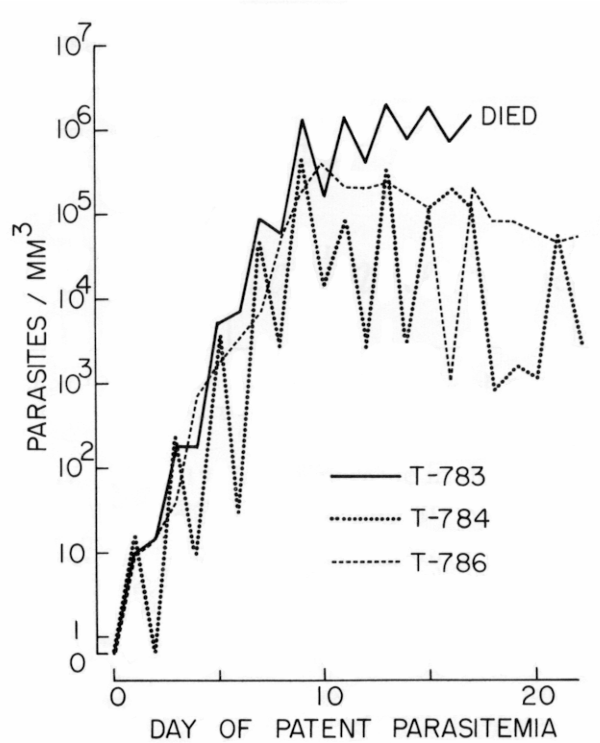


FIGURE 69.—Parasitemia of *Plasmodium fragile* in three *Macaca mulatta* monkeys infected via the bites of *Anopheles b. balabacensis* mosquitoes.

resulted during an observation period of 6 months. The sporozoites were proven infective when the infection in the control monkey became patent after 17 days.

The normal invertebrate host of *P. fragile* is not known. It is not unlikely that *Anopheles elegans* is the culprit in the Nilgiris because Choudhury *et al* (1963) incriminated it in the transmission of *P. cynomolgi* and *P. inui,* and probably the Nilgiri Parasite (= *P. fragile).* It will be recalled that the Nilgiri Parasite was first taken to be *P. inui.* However, until the natural vector is determined for the Nilgiris and for Ceylon, information on its vector potential and sporogonic cycle will have to be based on information derived from experimental vectors. We have infected *A. freeborni, A. b. balabacensis, A. maculatus,* and *A. quadrimaculatus* mosquitoes. Sporozoites were demonstrated in the salivary glands of only the first two. A comparison of the intensity of the infections (Table 39) shows that the most susceptible mosquito was *A. freeborni* followed by *A. b. balabacensis, A. maculatus,* and, finally, *A. quadrimaculatus*.

Immunity and Antigenic Relationships

There is not much one can say about antigenic relationships and immunity because of the paucity of information. About all that can be said at present is that *P. fragile* can exist as a mixed infection with *P. cynomolgi* in nature. Voller *et al* (1966) infected monkeys having a previous history of *P. cynomolgi, P. knowlesi,* and *P. coatneyi* infections with *P. fragile.* In the former, a normal infection developed, but in the monkey previously infected with *P. knowlesi,* there was a low parasitemia; in the monkey infected with *P. coatneyi,* there was complete immunity.

Serum samples from animals infected with *P. fragile* gave fluorescent antibody cross-reactions to *P. fieldi* at relatively high levels (mean reciprocal titer ratio of 100:93) and lower reactions to *P. gonderi* and *P. cynomolgi.* When the procedure was reversed, *P. fragile* antigen failed to cross-react at high levels with any of the 9 other simian antigens (Collins *et al,* 1966).

TABLE 39.—Comparative infectivity *of Plasmodium fragile* in *Anopheles freeborni, A. b. balabacensis, A. maculatus,* and *A. quadrimaculatus.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
F-1						100
F-1 : Bal	8	143	152	23.1	16.4	35.8
F-1 : Mac	6	79	123	53.2	4.1	2.2
F-1 : Q-1	5	54	36	74.1	2.8	0.06

* F-1 = *Anopheles freeborni,* Bal = *A. b. balabacensis,* Mac = *A. maculatus,* Q-1 = *A. quadrimaculatus.*
** GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of *A. freeborni* to another species where the GII of *A. freeborni* = 100.

REFERENCES

CHOUDHURY, D. S., WATTL, B. L. and RAMAKRISHNAN, S. P., 1963. Incrimination of *Anopheles elegans* James (1903) as a natural vector of simian malaria in the Nilgiris. Madras State, India. Ind. J. Malariol. *17* : 243-247.

COLLINS, W. E., SKINNER, J. C., and GUINN, E. G., 1966. Antigenic variations in the plasmodia of certain primates as detected by immuno-fluorescence. Am. J. Trop. Med. & Hyg. *15* : 483-485.

DISSANAIKE, A. S., NELSON, P. and GARNHAM, P. C. C., 1965. Two new malaria parasites, *Plasmodium cynomolgi ceylonensis* subsp. nov. and *Plasmodium fragile* sp. nov., from monkeys in Ceylon. Ceylon J. Med. Sci. *14* : 1-9.

DONOVAN, C., 1920. Malaria of monkeys. Ind. J. Med. Res. *7* : 717-721.

EYLES, D. E., COATNEY, G. R., and GETZ, M. E., 1960. Vivax-type malaria parasite of macaques transmissible to man. Science *131* : 1812-1813.

EYLES, D. E., 1963. The species of simian malaria: taxonomy, morphology, life cycle, and geographical distribution of the monkey species. J. Parasit. *49* : 866-887.

PRAKASH, S., and CHAKRABARTI, S. C., 1962. The isolation and description of *Plasmodium cynomolgi* (Mayer, 1907) and *Plasmodium inui* (Halberstadter and Prowazek, 1907) from naturally occurring mixed infections in *Macaca radiata radiata* monkeys of the Nilgiris, Madras State, India. Ind. J. Malariol. *16* : 303-311.

RAMAKRISHNAN, S. P. and MOHAN, B. N., 1961. Simian malaria in the Nilgiris, Madras State, India. Bull. Nat. Soc. Ind. Mal. Mosq. Dis. *9* : 139.

SINTON, J. A. and MULLIGAN, H. W., 1933. A critical review of the literature relating to the identification of the malarial parasites recorded from monkeys of the families Cercopithecidae and Colobidae. Rec. Malar. Surv. Ind. *III:* 381-443.

VOLLER, A., GARNHAM, P. C. C., and TARGETT, G. A. T., 1966. Cross immunity in monkey malaria. J. Trop. Med. Hyg. *69* : 121-123.